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Data Evaluation Report on the Acute Toxicity of BAS 800 H (Saflufenacil) to Aquatic Vascular Plants (*Lemna gibba*)

PMRA Submission Number: 2008-0431
PMRA Document ID: 1547234 EPA MRID Number: 47127922

Data Requirement:	PMRA DATA CODE	9.8.5
	EPA DP Barcode	DP349851
	OECD Data Point	IIA 8.6
	EPA MRID	47127922
	EPA Guideline	OPPTS 850.4400

Test material: BAS 800 H **Purity:** 93.9%
Common name: Saflufenacil
Chemical name: IUPAC: Not Reported
CAS name: N'-[2-chloro-4-fluoro-5-(3-methyl-2,6-dioxo-4-(trifluoromethyl)-3,6-dihydro-1(2H)-pyrimidinyl)benzoyl]-N-isopropyl-N-methylsulfamide
CAS No.: 372137-35-4
Synonyms: None Reported

Primary Reviewer: John Marton
Staff Scientist, Cambridge Environmental, Inc.
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Date: 03/25/08

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Date: 04/09/08

Primary Reviewer: Anita Pease
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Secondary Reviewer: Ann Lee
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Date: 06/09/09

Secondary Reviewer: Farzad Jahromi
DEWHA-APVMA
Date: 06/09/09

Company Code	BAZ
Active Code	SFF
Use Site Category:	13 (terrestrial feed crops) and 14 (terrestrial food crops)
EPA PC Code	118203

CITATION: Backfisch, K. 2006. Effect of BAS 800 H on the growth of *Lemna gibba*. Unpublished study performed by BASF Aktiengesellschaft, BASF Agricultural Center Limburgerhof, Crop Protection Division, Ecology and Environmental Analytics, Limburgerhof, Germany. Laboratory report number 2007/7013578. Study sponsored by BASF Corporation, Agricultural Products Division, Research Triangle Park, NC. Study completed June 8, 2006; report amended November 12, 2007

DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute toxicity of a pesticide to aquatic vascular plants. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

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EXECUTIVE SUMMARY:

In a 7-day acute toxicity study, freshwater floating aquatic vascular plants, duckweed (*Lemna gibba*), were exposed to BAS 800 H (Saflufenacil) at nominal concentrations of 0 (negative control), 1, 3.16, 10, 31.6, 100 and 316 µg/L under static-renewal conditions; the reviewer-calculated time-weighted average (TWA) concentrations were 0 (negative control), 2.01, 3.26, 10.2, 30.8, 93.9 and 298 µg a.i./L. The NOAEC, EC_{05} and EC_{50} values based on frond count were 10.2, 17 and 87 µg a.i./L, respectively. The percentage of growth inhibition, based on frond count, in the treated culture, as compared to the control, ranged from 2.6% to 89.0%.

Throughout the test, no compound-related phytotoxic effects were observed in the control or TWA 2.01-10.2 µg a.i./L treatment groups. Fronds in the TWA 30.8 µg a.i./L treatment group appeared smaller and partly brown on Day 7. Fronds in the TWA 93.9 µg a.i./L treatment group were partly brown on Day 5 and appeared smaller with shorter roots, and partly brown on Day 7. Fronds in the TWA 298 µg a.i./L treatment group were brown with loose roots on Days 3, 5 and 7.

This toxicity study is classified as **ACCEPTABLE** to the **U.S. EPA** and as **FULLY RELIABLE** to **PMRA and APVMA** as it is scientifically sound and satisfies the guideline requirement for an acute freshwater vascular plant toxicity study.

Results Synopsis

Test Organism: *Lemna gibba*
Test Type (Flow-through, Static, Static Renewal): Static-Renewal

Frond Count (Day 7):
EC_{05}: 17 µg a.i./L 95% C.I.: 13-22 µg a.i./L
EC_{50}: 87 µg a.i./L 95% C.I.: 78-97 µg a.i./L
NOAEC: 10.2µg a.i./L
Probit Slope: 2.32±0.123

Frond Count Growth Rate (Days 0-7):
EC_{05}: 40 µg a.i./L 95% C.I.: 35-45 µg a.i./L
EC_{50}: 140 µg a.i./L 95% C.I.: 130-140 µg a.i./L
NOAEC: 10.2µg a.i./L
Probit Slope: 3.08±0.0980

Dry Weight (Day 7):
EC_{05}: 13 µg a.i./L 95% C.I.: 7.9-23 µg a.i./L
EC_{50}: 95 µg a.i./L 95% C.I.: 78-120 µg a.i./L
NOAEC: 30.8µg a.i./L
Probit Slope: 1.93±0.187

Dry Weight Growth Rate (Days 0-7):
EC_{05}: 25 µg a.i./L 95% C.I.: 18-35 µg a.i./L
EC_{50}: >298 µg a.i./L 95% C.I.: N/A
NOAEC: 30.8µg a.i./L
Probit Slope: 1.49±0.104
Endpoint(s) Affected: Frond Count, Frond Count Growth Rate, Dry Weight, Dry Weight Growth Rate

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: This study was conducted following guidelines outlined in OECD draft guideline 221, "Lemna sp., Growth Inhibition Test"; and U.S. EPA, OPPTS 850.4400 (draft 1996). The following deviations from OPPTS 850.4400 were noted:

1. The pretest health of the duckweed culture was not reported.
2. The results of a periodic screening analysis of the dilution water were not provided.
3. OPPTS guidance requires that each replicate contain at least 12 fronds at test initiation; however, only 11 fronds were used to initiate the test. It should be noted, however, that the number of fronds used in the study meets the OECD Guideline 221, which specifies that the number of fronds per replicate should be between 9-12.
4. Analytical verification of the test material in the dilution water yielded recoveries of 367% of nominal for the lowest treatment level at test initiation and 303.6% of nominal on Day 3 prior to renewal of the solutions. The study author provided no justification for these high recoveries.

These deviations do not impact the acceptability of the study.

COMPLIANCE: Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided. This study was conducted in compliance with the OECD Principles of Good Laboratory Practice and the GLP Principles of the German "Chemikaliengesetz" (Chemicals Act) and meets the U.S. EPA Good Laboratory Practice Standards [40 CFR Part 160 (FIFRA) and Part 792 (TSCA)], with the exception that recognized differences exist between the GLP Principles/Standards of OECD and of FIFRA and TSCA.

A. MATERIALS:

1. Test material BAS 800 H (Saflufenacil)

Description: Solid White Powder

Lot No./Batch No. : COD000298 (Batch Number)

Purity: 93.9%

Stability of compound under test conditions: The reviewer-calculated TWA concentrations yielded recoveries of 93.9-201.4% of nominal.

(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)

Storage conditions of test chemicals: Not Reported

Physicochemical properties of BAS 800 H.

Parameter	Values	Comments
Water solubility at 20°C	0.21 mg/L	pH = 7

Parameter	Values	Comments
Vapor pressure	$>10^{-10}$ Pa (20/25°C)	
UV absorption	272 nm	pH1/pH7
pKa	Neutral	Ambient pH
Kow	Log P_{ow} 2.6	20°C

2. Test organism:

Name: Duckweed (*Lemna gibba*) *EPA requires a vascular species: Lemna gibba.*
Strain, if provided: G3
Source: In-house cultures
Age of inoculum: No more than 10 days old
Method of cultivation: 20X-AAP

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study: The study author reported that the nominal concentrations selected for the definitive test were based on the results of a range-finding test. However, the details and results from this test were not provided.

b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation period: Culturing media and conditions: (same as test or not) Health: (any mortality observed)	Continuous Same as test Not Reported	
Test system Static/static renewal Renewal rate for static renewal	Static-Renewal Test solutions were renewed on Days 3 and 5	*EPA expects the test concentrations to be renewed every 3 to 4 days (one renewal for the 7 day test, 3-4 renewals for the 14 day test).*

Parameter	Details	Remarks *Criteria*
Incubation facility	Temperature-controlled incubator equipped with several rotating trays at different heights and with fluorescent light tubes.	
Duration of the test	7 Days	*EPA requires a duration of 14 days. Seven day studies will be accepted for review by the Agency.*
Test vessel Material: *(glass/stainless steel)* Size: Fill volume:	 Glass 400 mL 160 mL	
Details of growth medium name pH at test initiation: pH at test termination: Chelator used: Carbon source:	20X-AAP 7.48-7.52 (New Solutions) 8.57-8.79 (Aged Solutions) Yes $NaHCO_3$	*EPA recommends the following culture media: Modified Hoagland's E+ or 20X-AAP. Chelating agents (e.g. EDTA) are recommended in the nutrient medium for optimum cell growth. Lower concentrations of chelating agents (down to one-third of the normal concentration recommended for AAP medium) may be used in the nutrient medium used for test solution preparation if it is suspected that the chelator will interact with the test material. ASTM reference, E1415-91and D 3978-80 (reapproved 1987).*
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	Yes	
Dilution water		

Parameter	Details	Remarks *Criteria*
source/type: pH: water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	Deioinzed water Adjusted to approximately 7.5 Millipore water (Milli Q system); filter-sterilized Not Reported Not Reported Not Reported Not Reported Not Reported	*EPA recommends a pH of ~5.0. A solution pH of 7.5 is acceptable if type 20X-AAP nutrient media is used.*
Indicate how the test material is added to the medium (added directly or used stock solution)	Serial dilution of a stock solution	
Aeration or agitation	Test vessels were placed on rotating trays; however, the RPM was not reported.	
Sediment used **(for rooted aquatic vascular plants)** Origin: Textural classification (%sand, silt, and clay): Organic carbon (%): Geographic location:	N/A	
Number of replicates Control: Solvent control: Treatments:	6 N/A 3/level	
Number of plants/replicate	3 plants per replicate	*EPA requires 5 plants.*
Number of fronds/plant	Two plants had four fronds each and one plant had three fronds, totaling 11 fronds per replicate.	*EPA requires 3 fronds per plant.*
Test concentrations Nominal:	0 (negative control), 1, 3.16, 10, 31.6, 100 and 316 µg/L	The measured concentrations in the DER represent the reviewer-calculated time-weighted average concentrations (see Appendix II).

Parameter	Details	Remarks *Criteria*
Mean-Measured: TWA-Measured	0 (<1.00), 2.47, 3.24, 10.11, 30.67, 94.77 and 300.14 µg a.i./L 0 (<1.00), 2.01, 3.26, 10.2, 30.8, 93.9 and 298 µg a.i./L	*EPA requires at least 5 test concentrations with a dose range of 2X or 3X progression.*
Solvent (type, percentage, if used)	N/A; a solvent was not used	
Method and interval of analytical verification	Samples were collected from fresh samples on Days 0, 3 and 5, while aged samples were collected on Days 3, 5 and 7. Samples were analyzed using HPLC with MS-detection.	
Test conditions Temperature: Photoperiod: Light intensity and quality:	 24.1-24.3°C Continuous illumination 6.91-9.05 klux	
Reference chemical (if used) name: concentrations:	 3-5 dichlorophenol N/A	A 7-day reference test was completed in February 2006 (BASF Doc ID2006/238318). Test concentrations were not provided.
Other parameters, if any	None	

2. Observations:

Table 2: Observation parameters

Parameters	Details	Remarks/Criteria
Parameters measured (e.g.,: number of fronds, plant dry weight or other toxicity symptoms)	-Frond Count -Growth Rate (Frond Count) -Dry Weight -Growth Rate (Dry Weight) -Phytotoxic Observations	
Measurement technique for frond number and other end points	Frond count and phytotoxic observations were made by visual inspection. The biomass based on the dry weight was	Biomass at test initiation was determined from a representative sample of the inoculum to allow for calculation of the growth rate based on

Parameters	Details	Remarks/Criteria
	determined using plant material from each replicate dried at 60°C to a constant weight. Growth rates were calculated based on the determined frond counts and dry weights.	dry weight. Every frond visibly projecting beyond the edge of the parent frond was counted.
Observation intervals	Frond count and phytotoxicity assessments were made on Days 3, 5 and 7. Growth rates were determined following test termination.	
Other observations, if any	None Reported	
Indicate whether there was an exponential growth in the control	Yes. By test termination, the mean frond count in the negative control was 145.5 fronds/rep.	
Were raw data included?	Yes	

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

Following 7 days of exposure, the mean frond count was 145.50 fronds/rep in the negative control and 137.33, 141.67, 139.67, 126.33, 64.33 and 16.00 fronds/rep in the TWA 2.01, 3.26, 10.2, 30.8, 93.9 and 298 µg a.i./L treatment groups, respectively, yielding inhibitions of 5.6, 2.6, 4.0, 13.2, 55.8 and 89.0%, respectively, relative to the negative control. The study author reported a NOAEC and EC_{50} values of 10.1 and 77.4 µg a.i./L, respectively, based on the mean-measured concentrations.

By test termination, the mean daily growth rate based on frond count was 0.369 fronds/day in the negative control and 0.360, 0.365, 0.363, 0.349, 0.252 and 0.053 fronds/day in the TWA 2.01, 3.26, 10.2, 30.8, 93.9 and 298 µg a.i./L treatment groups, respectively, yielding inhibitions of 2.4, 1.1, 1.6, 5.4, 31.7 and 85.6%, respectively, relative to the negative control. The study author reported a NOAEC and EC_{50} values of 10.1 and 143.6 µg a.i./L, respectively, based on the mean-measured concentrations.

By test termination, the mean dry weight was 15.975 mg in the negative control and 15.640, 16.480, 16.170, 15.070, 6.887 and 3.127 mg in the TWA 2.01, 3.26, 10.2, 30.8, 93.9 and 298 µg a.i./L treatment groups, respectively, yielding inhibitions of 2.1, -3.2, -1.2, 5.7, 56.9 and 80.4%, respectively, relative to the negative control. The study author reported a NOAEC and EC_{50} values of 30.7 and 98.6 µg a.i./L, respectively, based on the mean-measured concentrations.

By test termination, the mean daily growth rate based on dry weight was 0.494 mg/day in the negative control and 0.492, 0.499, 0.497, 0.486, 0.375 and 0.262 mg/day in the TWA 2.01, 3.26, 10.2, 30.8, 93.9 and 298 µg a.i./L treatment groups, respectively, yielding inhibitions of 0.4, -1.0, -0.6, 1.6, 24.1 and 47.0%, respectively, relative to the negative control. The study author reported a NOAEC and EC_{50} values of 30.7 and 294.2 µg a.i./L, respectively, based on the mean-measured concentrations.

Throughout the test, no compound-related phytotoxic effects were observed in the control or TWA 2.01-10.2 µg a.i./L treatment groups. Fronds in the TWA 30.8 µg a.i./L treatment group appeared smaller and partly brown on Day 7. Fronds in the TWA 93.9 µg a.i./L treatment group were partly brown on Day 5 and appeared smaller with shorter roots, and partly brown on Day 7. Fronds in the TWA 298 µg a.i./L treatment group were brown with loose roots on Days 3, 5 and 7.

Table 3: Effect of BAS 800 H (Saflufenacil) on frond number of *Lemna gibba* (Duckweed)

TWA and (Nominal) Concentrations µg a.i./L	Initial Frond Number/Rep	Mean Frond Number (per rep) at			
		Day 3	Day 5	Day 7	
				Frond Number	% Inhibition[1]
Negative control	11	27.50	64.50	145.50	N/A
2.01 (1)	11	27.33	59.33	137.33	5.6
3.26 (3.16)	11	27.67	60.33	141.67	2.6
10.2 (10)	11	28.00	59.00	139.67	4.0
30.8 (31.6)	11	27.33	59.00	126.33	13.2
93.9 (100)	11	24.67	39.00	64.33	55.8
298 (316)	11	13.67	15.00	16.00	89.0
Reference chemical (if used)	3,5-dichlorophenol. A reference test was completed in February 2006 (BASF Doc ID2006/238318) and yielded a 7-day EC_{50} (with 95% C.I.) for growth rate based on frond number of 11.29 mg/L (10.99-11.59 mg/L)				

[1] Inhibitions were reviewer-calculated
N/A- Not Applicable

Table 4: Effect of BAH 800 H (Saflufenacil) on growth of *Lemna gibba* (Duckweed)

TWA and (Nominal) Concentrations µg a.i./L	Mean Frond Growth Rate	Frond Growth Rate Inhibition[1] (%)	Mean Biomass Growth Rate	Biomass Growth Rate Inhibition[1] (%)	Mean Dry Weight (mg)	Dry Weight Inhibition[1] (%)
Negative control	0.369	N/A	0.494	N/A	15.975	N/A
2.01 (1)	0.360	2.4	0.492	0.4	15.640	2.1
3.26 (3.16)	0.365	1.1	0.499	-1.0	16.480	-3.2
10.2 (10)	0.363	1.6	0.497	-0.6	16.170	-1.2
30.8 (31.6)	0.349	5.4	0.486	1.6	15.070	5.7
93.9 (100)	0.252	31.7	0.375	24.1	6.887	56.9
298 (316)	0.053	85.6	0.262	47.0	3.127	80.4
Reference chemical	3,5-dichlorophenol. A reference test was completed in February 2006 (BASF Doc ID2006/238318) and yielded a 7-day EC_{50} (with 95% C.I.) for biomass based on dry weight of 7.81 mg/L (7.58-8.05 mg/L); the EC50 for growth rate based on biomass was 10.83 mg/L (10.57-11.09 mg/L)					

[1] Reviewer-estimated percent inhibition compared to the negative control. Negative percent inhibition indicates promoted growth.
N/A- not applicable

Table 5: Statistical endpoint values.

Statistical Endpoint	Frond No.	Frond Growth Rate	Dry Weight	Dry Weight Growth Rate
NOAEC or EC_{05} (µg a.i./L)	10.1	10.1	30.7	30.7
LOAEC (µg a.i./L)	30.7	30.7	94.8	94.8
IC_{50} or EC_{50} (µg a.i./L) (95% C.I.)	77.4 (73.6-81.4)	143.6 (137.2-150.4)	98.6 (93.6-103.9)	294.2 (270.9-319.6)
Other (IC_{10}/EC_{10})	14.0 (12.7-15.4)	34.5 (31.6-37.8)	27.5 (25.3-29.8)	60.4 (54.9-66.4)
Reference chemical (3,5-dichlorophenol) NOAEC (mg/L) IC_{50}/EC_{50} (mg/L)	NOAEC =1 EC_{50} = 8.02 (7.79-8.26)	NOAEC =1 EC_{50} = 11.29 (10.99-11.59)	NOAEC =1 EC_{50} = 7.81 (7.58-8.05)	NOAEC =1 EC_{50} = 10.83 (10.57-11.09)

* Do not use this table, if the study was deemed unacceptable.

B. REPORTED STATISTICS:

For each test concentration and for all growth parameters, means and standard deviations were calculated. The percent inhibition for all parameters relative to the control was determined and the respective concentration-response curves were drawn.

The NOAEC was determined using ANOVA followed by a Dunnett's/Bonferroni test. The ECx determination was done by probit, logit, or log-log model; the reported ECx values are based on the statistical model with the best fit. The calculations were conducted with a PC and the software package TOXSTAT 3.5.

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Prior to determining the toxicity values for frond count, frond count growth rate, dry weight and dry weight growth rate, the reviewer tested each data set for normality using the Chi-Square and Shapiro-Wilks tests and for homogeneity of variance using the Hartley and Bartlett's tests. If the data met these assumptions of ANOVA, the NOAEC value was determined using the parametric Bonferroni and Williams tests. If the data did not meet these assumptions, the NOAEC value was determined using the non-parametric Kruskal-Wallis test and direct observation of the dose-response data. NOAEC determinations were made using Toxstat statistical software. The ECx values, confidence intervals and slopes were determined using the probit analysis via Nuthatch statistical software. The reviewer-calculated TWA concentrations were used for all analyses.

Frond Count (Day 7):
EC_{05}: 17 µg a.i./L 95% C.I.: 13-22 µg a.i./L
EC_{50}: 87 µg a.i./L 95% C.I.: 78-97 µg a.i./L
NOAEC: 10.2µg a.i./L
Probit Slope: 2.32±0.123

Frond Count Growth Rate (Days 0-7):
EC_{05}: 40 µg a.i./L 95% C.I.: 35-45 µg a.i./L
EC_{50}: 140 µg a.i./L 95% C.I.: 130-140 µg a.i./L
NOAEC: 10.2µg a.i./L
Probit Slope: 3.08±0.0980

Dry Weight (Day 7):
EC_{05}: 13 µg a.i./L 95% C.I.: 7.9-23 µg a.i./L
EC_{50}: 95 µg a.i./L 95% C.I.: 78-120 µg a.i./L
NOAEC: 30.8µg a.i./L
Probit Slope: 1.93±0.187

Dry Weight Growth Rate (Days 0-7):
EC_{05}: 25 µg a.i./L 95% C.I.: 18-35 µg a.i./L
EC_{50}: >298 µg a.i./L 95% C.I.: N/A
NOAEC: 30.8µg a.i./L
Probit Slope: 1.49±0.104

D. STUDY DEFICIENCIES:

There were no study deficiencies.

E. REVIEWER'S COMMENTS:

The reviewer's results were based on the time-weighted average concentrations (refer to the associated Excel spreadsheet in Appendix II) while the study author's results were based on the mean-measured concentrations. Therefore, the reviewer's results are reported in the Executive Summary and Conclusions sections of this DER.

TWA concentrations were calculated using the following equation:

$$C_{TWA} = \frac{\left(\frac{C_1 + C_0}{2}\right)(t_1 - t_0) + \left(\frac{C_2 + C_1}{2}\right)(t_2 - t_1) + \left(\frac{C_{n-1} + C_2}{2}\right)(t_{n-1} - t_2) + \left(\frac{C_n + C_{n-1}}{2}\right)(t_n - t_{n-1})}{t_n}$$

where:
C_{TWA} is the time-weighted average concentration,
C_j is the concentration measured at time interval j (j = 0, 1, 2,...n)
t_j is the number of hours (or days or weeks, units used just need to be consistent in the equation) of the test at time interval j
(e.g., t_0 = 0 hours (test initiation), t_1 =48 hours, t_2 =96 hours)

Analytical verification of the test material in the dilution water yielded recoveries of 367% of nominal for the lowest treatment level at test initiation; all other levels yielded recoveries of 103.8-127.8% of nominal at test initiation. The lowest treatment level yielded a recovery of 303.6% of nominal on Day 3 prior to renewal of the solutions; all other treatment levels yielded recoveries of 87.7-95.6% of nominal. The study author provided no justification for the high recovery at the lowest level in the new and aged solutions on Days 0 and 3, respectively. The new solution at the lowest level yielded a recovery of 94.3% of nominal on Day 3, which was consistent with the recoveries obtained in the other new solutions on Day 3 (89.6-93.5% of nominal). The study authors determined the mean-measured concentrations for all levels, but excluded the measured concentrations from Days 0 and 3 for the lowest treatment level. However, the reviewer included these measured values in the time-weighted concentration as additional samples were analyzed and yielded similar results. Therefore, the reviewer's time-weighted average concentrations more closely represent the actual exposure concentrations.

The in-life portion of the definitive toxicity test was initiated on February 9, 2006 and completed on February 20, 2006.

F. CONCLUSIONS:

This study is scientifically sound and is classified as ACCEPTABLE to U.S. EPA and as FULLY RELIABLE to PMRA and APVMA. The NOAEC, EC_{05} and EC_{50} values based on frond count, the most sensitive endpoint, were 10.2, 17 and 87 µg a.i./L, respectively.

Frond Count (Day 7):
EC_{05}: 17 µg a.i./L 95% C.I.: 13-22 µg a.i./L
EC_{50}: 87 µg a.i./L 95% C.I.: 78-97 µg a.i./L
NOAEC: 10.2µg a.i./L
Probit Slope: 2.32±0.123

Frond Count Growth Rate (Days 0-7):
EC_{05}: 40 µg a.i./L 95% C.I.: 35-45 µg a.i./L
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EC_{05}: 13 µg a.i./L 95% C.I.: 7.9-23 µg a.i./L
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NOAEC: 30.8µg a.i./L
Probit Slope: 1.93±0.187

Dry Weight Growth Rate (Days 0-7):

EC_{05}: 25 µg a.i./L 95% C.I.: 18-35 µg a.i./L
EC_{50}: >298 µg a.i./L 95% C.I.: N/A
NOAEC: 30.8µg a.i./L
Probit Slope: 1.49±0.104
Endpoint(s) Affected: Frond Count, Frond Count Growth Rate, Dry Weight, Dry Weight Growth Rate

III. REFERENCES:

No references were provided.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

Frond count, Day 7; ug ai/L
File: 7922fc Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	1.608	5.808	9.168	5.808	1.608
OBSERVED	0	8	8	7	1

Calculated Chi-Square goodness of fit test statistic = 3.0586
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Frond count, Day 7; ug ai/L
File: 7922fc Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 950.833

W = 0.905

Critical W (P = 0.05) (n = 24) = 0.916
Critical W (P = 0.01) (n = 24) = 0.884

Data PASS normality test at P=0.01 level. Continue analysis.

Frond count, Day 7; ug ai/L
File: 7922fc Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 128.70
Closest, conservative, Table H statistic = 1705.0 (alpha = 0.01)

Used for Table H ==> R (# groups) = 7, df (# reps-1) = 2
Actual values ==> R (# groups) = 7, df (# avg reps-1) = 2.43
(average df used)

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal but do not differ greatly, the Hartley test may still be used as an approximate test (average df are used).

Frond count, Day 7; ug ai/L
File: 7922fc Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance

Calculated B statistic = 14.98
Table Chi-square value = 16.81 (alpha = 0.01)
Table Chi-square value = 12.59 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 2.43
Used for Chi-square table value ==> df (#groups-1) = 6

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

Frond count, Day 7; ug ai/L
File: 7922fc Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	48521.125	8086.854	144.586
Within (Error)	17	950.833	55.931	
Total	23	49471.958		

Critical F value = 2.70 (0.05,6,17)
Since F > Critical F REJECT Ho:All groups equal

Frond count, Day 7; ug ai/L
File: 7922fc Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	145.500	145.500		
2	2.01	137.333	137.333	1.544	
3	3.26	141.667	141.667	0.725	
4	10.2	139.667	139.667	1.103	
5	30.8	126.333	126.333	3.624	*
6	93.9	64.333	64.333	15.349	*
7	298	16.000	16.000	24.488	*

Bonferroni T table value = 2.65 (1 Tailed Value, P=0.05, df=17,6)

Frond count, Day 7; ug ai/L
File: 7922fc Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	neg control	6			
2	2.01	3	14.040	9.6	8.167
3	3.26	3	14.040	9.6	3.833
4	10.2	3	14.040	9.6	5.833
5	30.8	3	14.040	9.6	19.167
6	93.9	3	14.040	9.6	81.167
7	298	3	14.040	9.6	129.500

Frond count, Day 7; ug ai/L
File: 7922fc Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	6	145.500	145.500	145.500
2	2.01	3	137.333	137.333	139.556
3	3.26	3	141.667	141.667	139.556
4	10.2	3	139.667	139.667	139.556
5	30.8	3	126.333	126.333	126.333
6	93.9	3	64.333	64.333	64.333
7	298	3	16.000	16.000	16.000

Frond count, Day 7; ug ai/L
File: 7922fc Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
neg control	145.500				
2.01	139.556	1.124		1.74	k= 1, v=17
3.26	139.556	1.124		1.82	k= 2, v=17
10.2	139.556	1.124		1.85	k= 3, v=17
30.8	126.333	3.624	*	1.87	k= 4, v=17
93.9	64.333	15.348	*	1.87	k= 5, v=17
298	16.000	24.488	*	1.88	k= 6, v=17

s = 7.479
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	17.	13.	22.	0.054	0.77
EC10	24.	20.	30.	0.046	0.80
EC25	45.	38.	52.	0.034	0.85
EC50	87.	78.	97.	0.022	0.90

Slope = 2.32 Std.Err. = 0.123

Goodness of fit: p = 0.36 based on DF= 4.0 17.

7922FC : Frond count, Day 7; ug ai/L

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	146.	143.	2.61	100.	0.00
2.01	3.00	137.	143.	-5.55	100.	0.00740
3.26	3.00	142.	143.	-1.16	100.	0.0471
10.2	3.00	140.	141.	-1.02	98.5	1.54
30.8	3.00	126.	122.	4.57	85.2	14.8
93.9	3.00	64.3	67.1	-2.72	46.9	53.1
298.	3.00	16.0	15.4	0.645	10.7	89.3

Frond growth rate (average daily rate),Days0-7; ug ai/L
File: 7922fr Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	1.608	5.808	9.168	5.808	1.608
OBSERVED	0	8	8	7	1

Calculated Chi-Square goodness of fit test statistic = 3.0586
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Frond growth rate (average daily rate),Days0-7; ug ai/L
File: 7922fr Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 0.001

W = 0.957

```
Critical W (P = 0.05) (n = 24) = 0.916
Critical W (P = 0.01) (n = 24) = 0.884
--------------------------------------------------------------------------------

Data PASS normality test at P=0.01 level. Continue analysis.


Frond growth rate (average daily rate),Days0-7; ug ai/L
File: 7922fr        Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance
--------------------------------------------------------------------------------

Calculated H statistic (max Var/min Var) =   51.71
Closest, conservative, Table H statistic = 1705.0  (alpha = 0.01)

Used for Table H ==>     R (# groups) =   7,    df (# reps-1) =       2
Actual values    ==>     R (# groups) =   7,    df (# avg reps-1) =   2.43
                                                (average df used)

--------------------------------------------------------------------------------

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal
      but do not differ greatly, the Hartley test may still be used
      as an approximate test (average df are used).


Frond growth rate (average daily rate),Days0-7; ug ai/L
File: 7922fr       Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance
--------------------------------------------------------------------------------

Calculated B statistic =    6.43
Table Chi-square value =   16.81  (alpha = 0.01)
Table Chi-square value =   12.59  (alpha = 0.05)

Average df used in calculation  ==>   df (avg n - 1) =   2.43
Used for Chi-square table value ==>   df (#groups-1) =   6
--------------------------------------------------------------------------------

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is
      used to calculate the B statistic (see above).

Frond growth rate (average daily rate),Days0-7; ug ai/L
File: 7922fr        Transform: NO TRANSFORMATION

                              ANOVA TABLE
--------------------------------------------------------------------------------

SOURCE            DF              SS                MS            F
--------------------------------------------------------------------------------
```

Between	6	0.2574	0.0429	429.000
Within (Error)	17	0.0011	0.0001	
Total	23	0.2585		

Critical F value = 2.70 (0.05,6,17)
Since F > Critical F REJECT Ho:All groups equal

Frond growth rate (average daily rate),Days0-7; ug ai/L
File: 7922fr Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	0.368	0.368		
2	2.01	0.360	0.360	1.131	
3	3.26	0.365	0.365	0.519	
4	10.2	0.363	0.363	0.801	
5	30.8	0.349	0.349	2.781	*
6	93.9	0.252	0.252	16.405	*
7	298	0.053	0.053	44.548	*

Bonferroni T table value = 2.65 (1 Tailed Value, P=0.05, df=17,6)

Frond growth rate (average daily rate),Days0-7; ug ai/L
File: 7922fr Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	neg control	6			
2	2.01	3	0.019	5.1	0.008
3	3.26	3	0.019	5.1	0.004
4	10.2	3	0.019	5.1	0.006
5	30.8	3	0.019	5.1	0.020
6	93.9	3	0.019	5.1	0.116
7	298	3	0.019	5.1	0.315

Frond growth rate (average daily rate),Days0-7; ug ai/L
File: 7922fr Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN

1	neg control	6	0.368	0.368	0.368
2	2.01	3	0.360	0.360	0.363
3	3.26	3	0.365	0.365	0.363
4	10.2	3	0.363	0.363	0.363
5	30.8	3	0.349	0.349	0.349
6	93.9	3	0.252	0.252	0.252
7	298	3	0.053	0.053	0.053

Frond growth rate (average daily rate),Days0-7; ug ai/L
File: 7922fr Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
neg control	0.368				
2.01	0.363	0.977		1.74	k= 1, v=17
3.26	0.363	0.977		1.82	k= 2, v=17
10.2	0.363	0.977		1.85	k= 3, v=17
30.8	0.349	3.324	*	1.87	k= 4, v=17
93.9	0.252	19.608	*	1.87	k= 5, v=17
298	0.053	53.245	*	1.88	k= 6, v=17

s = 0.008
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	40.	35.	45.	0.024	0.89
EC10	52.	47.	58.	0.021	0.90
EC25	82.	76.	88.	0.015	0.93
EC50	1.4E+02	1.3E+02	1.4E+02	0.010	0.95

Slope = 3.08 Std.Err. = 0.0980

Goodness of fit: p = 0.65 based on DF= 4.0 17.

7922FR : Frond growth rate (average daily rate),Days0-7; ug ai/L

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	0.368	0.364	0.00452	100.	0.00
2.01	3.00	0.360	0.364	-0.00348	100.	8.95e-07
3.26	3.00	0.365	0.364	0.000850	100.	3.10e-05
10.2	3.00	0.363	0.364	-0.00105	100.	0.0269
30.8	3.00	0.349	0.355	-0.00656	97.6	2.36
93.9	3.00	0.252	0.251	0.00144	69.0	31.0
298.	3.00	0.0533	0.0536	-0.000232	14.7	85.3

Frond dry weight (mg), Day 7; ug ai/L
File: 7922dw Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	1.608	5.808	9.168	5.808	1.608
OBSERVED	0	8	9	6	1

Calculated Chi-Square goodness of fit test statistic = 2.6746
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Frond dry weight (mg), Day 7; ug ai/L
File: 7922dw Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 19.633

W = 0.864

Critical W (P = 0.05) (n = 24) = 0.916
Critical W (P = 0.01) (n = 24) = 0.884

Data FAIL normality test. Try another transformation.

Warning - The two homogeneity tests are sensitive to non-normal data and should not be performed.

Frond dry weight (mg), Day 7; ug ai/L
File: 7922dw Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 140.65
Closest, conservative, Table H statistic = 1705.0 (alpha = 0.01)

Used for Table H ==> R (# groups) = 7, df (# reps-1) = 2
Actual values ==> R (# groups) = 7, df (# avg reps-1) = 2.43
(average df used)

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal

but do not differ greatly, the Hartley test may still be used
as an approximate test (average df are used).

Frond dry weight (mg), Day 7; ug ai/L
File: 7922dw Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance

Calculated B statistic = 14.27
Table Chi-square value = 16.81 (alpha = 0.01)
Table Chi-square value = 12.59 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 2.43
Used for Chi-square table value ==> df (#groups-1) = 6

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is
used to calculate the B statistic (see above).

Frond dry weight (mg), Day 7; ug ai/L
File: 7922dw Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	557.255	92.876	80.412
Within (Error)	17	19.633	1.155	
Total	23	576.888		

Critical F value = 2.70 (0.05,6,17)
Since F > Critical F REJECT Ho:All groups equal

Frond dry weight (mg), Day 7; ug ai/L
File: 7922dw Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	15.975	15.975		
2	2.01	15.640	15.640	0.441	
3	3.26	16.480	16.480	-0.665	
4	10.2	16.170	16.170	-0.257	
5	30.8	15.070	15.070	1.191	
6	93.9	6.887	6.887	11.959	*
7	298	3.127	3.127	16.907	*

Bonferroni T table value = 2.65 (1 Tailed Value, P=0.05, df=17,6)

Frond dry weight (mg), Day 7; ug ai/L
File: 7922dw Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	neg control	6			
2	2.01	3	2.018	12.6	0.335
3	3.26	3	2.018	12.6	-0.505
4	10.2	3	2.018	12.6	-0.195
5	30.8	3	2.018	12.6	0.905
6	93.9	3	2.018	12.6	9.088
7	298	3	2.018	12.6	12.848

Frond dry weight (mg), Day 7; ug ai/L
File: 7922dw Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	6	15.975	15.975	16.048
2	2.01	3	15.640	15.640	16.048
3	3.26	3	16.480	16.480	16.048
4	10.2	3	16.170	16.170	16.048
5	30.8	3	15.070	15.070	15.070
6	93.9	3	6.887	6.887	6.887
7	298	3	3.127	3.127	3.127

Frond dry weight (mg), Day 7; ug ai/L
File: 7922dw Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
neg control	16.048				
2.01	16.048	0.096		1.74	k= 1, v=17
3.26	16.048	0.096		1.82	k= 2, v=17
10.2	16.048	0.096		1.85	k= 3, v=17
30.8	15.070	1.191		1.87	k= 4, v=17
93.9	6.887	11.960	*	1.87	k= 5, v=17
298	3.127	16.908	*	1.88	k= 6, v=17

s = 1.075
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	13.	7.9	23.	0.11	0.59
EC10	21.	13.	33.	0.094	0.64
EC25	43.	31.	59.	0.067	0.73
EC50	95.	78.	1.2E+02	0.041	0.82

Slope = 1.93 Std.Err. = 0.187

!!!Poor fit: p = 0.0067 based on DF= 4.0 17.

7922DW : Frond dry weight (mg), Day 7; ug ai/L

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	16.0	16.3	-0.302	100.	0.00
2.01	3.00	15.6	16.3	-0.627	99.9	0.0603
3.26	3.00	16.5	16.2	0.241	99.8	0.231
10.2	3.00	16.2	15.8	0.388	97.0	3.04
30.8	3.00	15.1	13.5	1.58	82.9	17.1
93.9	3.00	6.89	8.23	-1.34	50.5	49.5
298.	3.00	3.13	2.77	0.362	17.0	83.0

Dry weight growth rate (avg. daily rate), D 0-7;ug ai/L
File: 7922wr Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	1.608	5.808	9.168	5.808	1.608
OBSERVED	1	7	8	7	1

Calculated Chi-Square goodness of fit test statistic = 1.0979
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Dry weight growth rate (avg. daily rate), D 0-7;ug ai/L
File: 7922wr Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 0.002

```
W =    0.913

Critical W (P = 0.05) (n = 24) = 0.916
Critical W (P = 0.01) (n = 24) = 0.884
-------------------------------------------------------------------------------

Data PASS normality test at P=0.01 level. Continue analysis.


Dry weight growth rate (avg. daily rate), D 0-7;ug ai/L
File: 7922wr        Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance
-------------------------------------------------------------------------------

Calculated H statistic (max Var/min Var) =   20.93
Closest, conservative, Table H statistic = 1705.0  (alpha = 0.01)

Used for Table H ==>     R (# groups) =   7,    df (# reps-1) =       2
Actual values    ==>     R (# groups) =   7,    df (# avg reps-1) =   2.43
                                                (average df used)

-------------------------------------------------------------------------------

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal
      but do not differ greatly, the Hartley test may still be used
      as an approximate test (average df are used).


Dry weight growth rate (avg. daily rate), D 0-7;ug ai/L
File: 7922wr       Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance
-------------------------------------------------------------------------------

Calculated B statistic =    6.31
Table Chi-square value =   16.81  (alpha = 0.01)
Table Chi-square value =   12.59  (alpha = 0.05)

Average df used in calculation  ==>   df (avg n - 1) =   2.43
Used for Chi-square table value ==>   df (#groups-1) =   6
-------------------------------------------------------------------------------

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is
      used to calculate the B statistic (see above).

Dry weight growth rate (avg. daily rate), D 0-7;ug ai/L
File: 7922wr        Transform: NO TRANSFORMATION

                                ANOVA TABLE
-------------------------------------------------------------------------------
```

SOURCE	DF	SS	MS	F
Between	6	0.1582	0.0264	264.000
Within (Error)	17	0.0018	0.0001	
Total	23	0.1600		

Critical F value = 2.70 (0.05,6,17)
Since F > Critical F REJECT Ho:All groups equal

Dry weight growth rate (avg. daily rate), D 0-7;ug ai/L
File: 7922wr Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	0.494	0.494		
2	2.01	0.492	0.492	0.354	
3	3.26	0.499	0.499	-0.731	
4	10.2	0.497	0.497	-0.354	
5	30.8	0.487	0.487	1.061	
6	93.9	0.375	0.375	16.900	*
7	298	0.262	0.262	32.880	*

Bonferroni T table value = 2.65 (1 Tailed Value, P=0.05, df=17,6)

Dry weight growth rate (avg. daily rate), D 0-7;ug ai/L
File: 7922wr Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	neg control	6			
2	2.01	3	0.019	3.8	0.003
3	3.26	3	0.019	3.8	-0.005
4	10.2	3	0.019	3.8	-0.002
5	30.8	3	0.019	3.8	0.007
6	93.9	3	0.019	3.8	0.119
7	298	3	0.019	3.8	0.233

Dry weight growth rate (avg. daily rate), D 0-7;ug ai/L
File: 7922wr Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	ORIGINAL	TRANSFORMED	ISOTONIZED

	IDENTIFICATION	N	MEAN	MEAN	MEAN
1	neg control	6	0.494	0.494	0.495
2	2.01	3	0.492	0.492	0.495
3	3.26	3	0.499	0.499	0.495
4	10.2	3	0.497	0.497	0.495
5	30.8	3	0.487	0.487	0.487
6	93.9	3	0.375	0.375	0.375
7	298	3	0.262	0.262	0.262

Dry weight growth rate (avg. daily rate), D 0-7;ug ai/L
File: 7922wr Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
neg control	0.495				
2.01	0.495	0.146		1.74	k= 1, v=17
3.26	0.495	0.146		1.82	k= 2, v=17
10.2	0.495	0.146		1.85	k= 3, v=17
30.8	0.487	1.061		1.87	k= 4, v=17
93.9	0.375	16.900	*	1.87	k= 5, v=17
298	0.262	32.880	*	1.88	k= 6, v=17

s = 0.010
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	25.	18.	35.	0.071	0.71
EC10	44.	34.	57.	0.055	0.77
EC25	1.1E+02	97.	1.3E+02	0.030	0.86
EC50	3.2E+02	2.9E+02	3.5E+02	0.022	0.90

Slope = 1.49 Std.Err. = 0.104

!!!Poor fit: p = 0.0024 based on DF= 4.0 17.

7922WR : Dry weight growth rate (avg. daily rate), D 0-7;ug ai/L

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	0.494	0.498	-0.00424	100.	0.00
2.01	3.00	0.492	0.498	-0.00647	99.9	0.0531
3.26	3.00	0.499	0.498	0.00169	99.8	0.153
10.2	3.00	0.497	0.492	0.00479	98.7	1.31
30.8	3.00	0.487	0.466	0.0210	93.4	6.56
93.9	3.00	0.375	0.391	-0.0165	78.5	21.5

298.	3.00	0.262	0.258	0.00404	51.7	48.3

!!!Warning: EC50 not bracketed by doses evaluated.

APPENDIX II: COPY OF REVIEWER'S TWA CALCULATIONS:

Time-Weighted Average Concentrations

Nominal (µg/L)	Day 0-New	% of Nominal	Day 3-Aged	% of New	Day 3-New	% of Nominal	Day 5-Aged	% of New	Day 5-New	% of Nominal	Day Ag
Negative Control	0	N/A	0	N/A	0	N/A	0	N/A	0	N/A	0
1	3.67	367.0	3.04	82.8	0.943	94.3	0.837	88.8	1.23	123.0	1.0
3.16	4.04	127.8	2.83	70.0	2.89	91.5	2.6	90.0	3.98	125.9	3.0
10	12.2	122.0	9.6	78.7	9.32	93.2	8.1	86.9	12.2	122.0	9.2
31.6	36.9	116.8	25.8	69.9	29.2	92.4	25.3	86.6	38	120.3	28
100	107	107.0	71.7	67.0	93.5	93.5	80.6	86.2	122	122.0	93
316	328	103.8	242	73.8	283	89.6	260	91.9	382	120.9	30

Time-Weighted Average Concentrations

Nominal (µg/L)	% Inhibition
Negative Control	N/A
1	0.4
3.16	-1.0
10	-0.6
31.6	1.6
100	24.1
316	47.0